CURRENT LITERATURE

NOTES FOR STUDENTS

Formation and translocation of carbohydrates in plants.—In a series of three papers from the Rothamsted Experimental Station, DAVIS, DAISH, and SAWYER [1, 2, 3] have reported the results of an investigation designed to test the validity of BROWN and MORRIS' conclusion that cane sugar is the primary photosynthetic product in foliage leaves, that the dextrose and levulose present are products of its hydrolysis, not its precursors, and that levulose is found in excess in the leaves and leaf stalks for the reason that dextrose is more readily utilized in respiration. The introductory review of literature presents an account of work done in this field since the appearance of the memoir by BROWN and MORRIS in 1893. The workers who have given attention to the subject since that time fall into three groups: WENT, STROHMER, STEPHANI, PEKLO, and PARKIN, who adhere to the view that saccharose is the first sugar formed in photosynthesis; MAQUENNE, STRAKOSCH, ROBERTSON, IRVINE and DOBSON, GUTZEIT, and DELEANO, who consider that hexoses are the primary product; and PELLET and COLIN, who hold the belief that saccharose, dextrose, and levulose are formed simultaneously in the leaf and transported as such to the storage organs, where conversion of the reducing sugars into saccharose subsequently occurs.

The authors made analyses of leaves, midribs, and upper and lower halves of petioles of Yellow Globe mangold at three stages of growth: an early stage (August 26) when leaf formation was predominant, the seeds having been sown June 9; an intermediate stage (September 10) when leaf growth had practically ceased and storage of sugar in the root had attained its maximum rate; and a final stage (October 11) when growth of roots had been practically completed. Samples were collected at intervals of 2 hours over a 24 hour period on each

[1] DAVIS, WILLIAM A., DAISH, ARTHUR JOHN, and SAWYER, GEORGE CONWORTH, Studies of the formation and translocation of carbohydrates in plants. I. The carbohydrate of the mangold leaf. Jour. Agric. Sci. 7:255–326. 1916.

[2] DAVIS, WILLIAM A., Studies of the formation and translocation of carbohydrates in plants. II. The dextrose-levulose ratio in the mangold. Jour. Agric. Sci. 7:327–351. 1916.

[3] DAVIS, WILLIAM A., and SAWYER, GEORGE CONWORTH, Studies of the formation and translocation of carbohydrates in plants. III. The carbohydrates of the leaf and leaf stalks of the potato. The mechanism of degradation of starch in the potato. Jour. Agric. Sci. 7:352–384. 1916.

of the dates given. Chemical changes subsequent to collection were prevented by dropping the material immediately into a large volume of boiling 95 per cent alcohol containing 1 per cent concentrated ammonia, which instantly destroys the enzymes present. In the subsequent analyses, the methods outlined by the authors in their papers on the estimation of carbohydrates in plant material were employed; the chief new features of these methods are the employment of 10 per cent citric acid for the inversion of saccharose, the estimation of maltose by fermentation with maltase-free yeasts, and the determination of starch by the use of taka-diastase.

Maltose and starch were entirely absent from the leaves at all times, day and night, in all three series. Starch is present in very young leaves, but disappears as soon as the roots have grown sufficiently to be capable of storing sugar. In the first series, hexoses began to increase immediately after sunrise, attained a maximum of 2.16 per cent between 10 A.M. and noon, declined sharply until 4 P.M., then decreased steadily throughout the night to start upward again at 4 A.M. The curve representing saccharose rose more slowly from sunrise, maintaining a maximum of 3.11 to 3.06 per cent from noon to 4 P.M., then dropped in an almost straight line through the night to start up at 4 A.M. Both curves roughly paralleled the temperature curve.

In the second series (that of September 10) both curves were complicated; that for hexose shows a minimum at 8 A.M., with a rapid rise to 7.5 per cent at 1 P.M., followed by a slight decline for 3 hours which is succeeded by a rise to 8.9 per cent at 6 P.M. Two hours later this has fallen to 6.75 per cent, but there is again a rise to a new but lower maximum of 7.81 per cent at 2 A.M., after which there is a sharp decline, continuing until sunrise. The curve for saccharose is similar, in that it shows two maxima at 6 P.M. and 2 A.M., but differs in that the second is much the largest, the amounts being 6.39 and 8.27 per cent. The curves for hexose and saccharose in the third series are alike in that each presents three maxima; for hexose these occur at 1 P.M., 9 P.M., and 3 A.M., the last being greatest, while those for saccharose occur at 3 P.M., 9 P.M., and 3 A.M., the second being considerably higher than the others. In neither the second nor the third series is there any resemblance to the temperature curve. In the first series, the amount of saccharose present is at all times much greater than that of hexose, becoming 7 times as great at 4 A.M., and the fluctuations in amount of hexoses are much greater than those of saccharose. In the second series, hexoses vary between 8.9 and 5.4 per cent and are larger in amount than saccharose, which varies from 8.27 to 4.24 per cent. To this statement there is one exception at 2 A.M., at which hour saccharose is slightly in excess. In the third series, hexoses are again in excess, varying from 12.41 to 9.39 per cent, while saccharose ranges between 9.52 and 4.98 per cent. The variations in saccharose are greater than those in dextrose in both second and third series, and are greater in the third than in the second. Consequently, while the ratio of invert sugar to cane sugar varies, in the first series, between 0.133 (at 4 A.M.) and 0.710 (at 10 A.M.), and is expressed by a curve closely paralleling the temperature curve, the ratio for the second series has a maximum of 1.60 at 4 P.M. and exceeds unity at all hours except at 2 A.M., when it drops to 0.94, but still roughly parallels the temperature curve. In the final series the ratio ranges between 1.93 at 7 A.M. and 1.14 at 3 P.M. as extremes.

For total sugars (hexoses plus saccharoses) the maximum in the August 26 series is 5.26 per cent, reached at 12 noon; the minimum, 1.70 per cent, is attained at 4 A.M.. On September 11 the maximum of 16.08 per cent is attained at 4 A.M., the minimum of 9.98 per cent at 8 A.M., with a second rise to 15.29 per cent at 6 P.M. On October 11 the maximum of 20.99 per cent occurs at 7 P.M., is followed by a slightly lower maximum at 3 A.M., with the minimum, 14.5 per cent, occurring at 7 A.M.

In the first series, pentoses vary during the daylight hours only between 0.37 and 0.45 per cent, dropping again to 0.36 at 8 P.M., only to rise slowly through the night to 0.52 at 4 A.M. Pentosans remain practically constant through the day in the neighborhood of 5.5 per cent, with a maximum of 5.96 at 4 P.M. In the second series, the fluctuations in pentose have much wider limits; there is increase from 0.34 to 0.68 per cent between 10 A.M. and 2 P.M., followed by a fall to 0.45 at sunset and a subsequent rapid rise to 0.71, remaining stationary through the dark hours and dropping suddenly to 0.5 at 4 A.M. Pentosans rise slightly between noon and 2 P.M., then remain stationary until 4 A.M., when there is a second slight rise, but the fluctuations are between 4.42 and 5.9 per cent as extremes. In the final series, pentoses remain almost unchanged at 0.9 per cent from 9 A.M. until 9 P.M., declining to a minimum of 0.61 per cent at sunrise (7 A.M.). Pentosans here show very slight fluctuations, but are slightly higher (6.77 to 7.15 per cent) during darkness than in the day (6.21 to 6.55 per cent). The total percentage of material soluble in alcohol falls slowly throughout the day in the first series, attaining a minimum at 4 P.M., then remains nearly constant through the night. In the second series there is a decline in alcohol-soluble constituents from 4 A.M. to 1 P.M., then a rise continuing until sunset, with a drop between 6 and 8 P.M., then a very slow rise from 47.2 to 51.3 per cent between 8 P.M. and 4 A.M. In the third series the percentages of alcohol-soluble materials are almost constant from 7 A.M. to 9 P.M., varying only from 52.0 to 54.9 per cent, then run down at 11 P.M. to 47.9 per cent, only to return at the next sampling to the general level.

The increases in pentosans throughout the day in the first and second series are attributed in part to the building of new ligneous tissue, in part to the formation of gums which play the rôle of reserves. In the third series, when the leaves have ceased to grow, the fluctuations are apparent rather than real, being due to fluctuations in total sugars. The striking feature of the curves for sugar are the two night maxima which occur in both second and third series, since both hexoses and saccharose increase synchronously to a point higher than that reached during insolation, their sum total also exceeding that attained in the day and reaching its greatest amount at the same time, between midnight and 3 A.M, in both series, so that the results cannot be due to interconversion.

In the entire absence of both maltose and starch, the authors attribute this increase to the conversion into saccharose and invert sugar of some gummy substance, which is present in large amounts and which is precipitated in semi-crystalline form by basic lead acetate after treatment with taka-diastase. There is also an interconnection between the fall and rise of pentoses which occurs between 4 and 8 P.M., and the change in the opposite direction in saccharose and hexoses.

While pentosans make up 8.58 to 9.61 per cent of the insoluble matter of the leaves in the first series, there is an increase to 9.83–10.85 per cent in the second and a further increase to 13.70–15.35 in the third. While in the first series about one-half the saccharose and nearly all the hexoses are used up during the night, the second and third series show a very much larger amount of reducing sugars present at the beginning of the day, and the amount of these up to the attainment of the first maximum is always greater than that of saccharose, but when root growth is nearly complete, as in the third series, the range of variation in cane sugar in the leaf becomes much greater, the leaf apparently acting as a storage reservoir during insolation. The range of variations during growth is summarized as follows:

Series and date	Insoluble in alcohol	Saccharose	Hexoses
I, August 26........	57.5–62.9	1.50–3.11	0.20– 2.16
II, September 10....	45.3	4.24–8.27	5.38– 8.90
III, October 11	55.8	4.98–9.52	9.39–12.41

Series and date	Pentose	Pentosans	Ratio, invert to saccharose	Total saccharose plus hexose
I, August 26........	0.36–0.52	5.19–5.96	0.13– .71	1.70– 5.20
II, September 10....	0.34–0.76	4.42–5.90	0.94–1.60	9.98–16.08
III, October 11	0.61–0.92	6.21–7.15	1.14–1.93	14.50–20.99

Information as to the translocation of the sugars was obtained by making separate analyses of the midribs and petioles. At any given picking the amount of sugars and alcohol soluble matter is always greater in midribs than in leaves, greater in top halves of petioles than in midribs, and greater in lower halves than in top halves of petioles. In the first series, the total amount of hexoses and of apparent levulose in the stalks increases very rapidly during the forenoon to reach a maximum at noon, while the corresponding increases in dextrose and saccharose are extremely slight, dextrose being actually larger in amount at midnight than at any time during the day. In the bottom halves of the petioles of the series, total hexose, apparent dextrose, and apparent levulose run very closely together, reaching a maximum at noon, declining steadily to midnight, and then separating, as levulose continues to decline while the others start upward again. Saccharose rises slightly from 6 A.M. to noon, and then remains stationary for the succeeding 18 hours. In the second series, in which top and bottom halves of petioles were not analyzed separately, saccharose was constant throughout the 24 hours; total hexoses and apparent levulose were least at 11 P.M., increased slowly to 4 A.M., then more rapidly until 4 P.M., when they again declined together until 11 P.M. Apparent dextrose rose from 4 A.M. until 4 P.M., then remained stationary throughout afternoon and night. In the midribs, however, total hexoses decreased slowly from 10 A.M. to 4 P.M., then more rapidly through the night, rising again at 4 A.M. Apparent levulose decreased, apparent dextrose increased, from 10 A.M. to 4 P.M., after which dextrose rather rapidly fell off while levulose slowly increased until 4 A.M., when both began to increase. Saccharose was stationary from 10 A.M. to 4 P.M., then increased slowly and uniformly through the evening and night, beginning to fall at 4 A.M. There is, therefore, a steady movement of sugars from leaves to midribs, thence through the stalks, the maximum in leaves at 2 A.M. moving onward into the stalks to give a maximum there at 6 A.M., which is succeeded by a minimum 4 hours later, when a large part of the sugar formed during the insolation of the preceding day has passed from stalk to root. The ratios of invert sugar to cane sugar at any given hour of the day, as at 6 A.M., September 10, when it is 1.48 in leaf, 3.32 in midrib, and 5.27 in stalk, are significant, showing as they do that there are progressively more and more hexoses in the stream of sugars as it passes from leaf to root. On August 26 the stalks had at noon 4.25 per cent saccharose and 11.57 per cent hexose; at 10 A.M., September 10, 4.82 per cent saccharose and 20.5 per cent hexose; and at 11 A.M., October 11, 5.29 per cent saccharose and 25.7 per cent hexose. This is strong evidence that hexoses are translocation forms produced by the conversion of cane sugar, as is the fact that cane sugar greatly predominates in the leaves in the early stages of growth, prior to the beginning of storage in the roots. Further evidence is seen in the fact that cane sugar is the predominant sugar in the leaves of the potato, vine, and snowdrop, although these plants store carbohydrate as starch, dextrose, and inulin respectively, and do not store cane sugar. Cane sugar is therefore formed in the mesophyll, transported into the vessels, undergoes progressive inversion as it passes onward through midribs and stalks, enters the roots as reducing sugars, and these are there transformed once more into saccharose. The authors have not studied the mechanism of this synthesis in the root; invertase was shown to be present in the sieve tubes but was not found in roots by ROBERTSON, IRVINE, and DOBSON, and it is believed to be the agent in the inversion occurring during transport. Since the existence of the saccharogenic enzyme of BORDET has not yet been substantiated, and the probability of reversible zymohydrolysis by invertase is contra-indicated by the absence of invertase from the roots, the authors are unable to formulate a theory as to the agent responsible for this synthesis.

In the second paper of the series DAVIS reports the result of a study of the dextrose-levulose ratio in the mangold. The determination of these sugars by polarimetric methods is falsified by the presence of optically active substances not precipitable by basic lead acetate. Glutamine, glutaminic acid, and aspartic acid give a dextro-rotation which is increased by acids, while asparagine gives either dextro- or laevo-rotation accordingly as the solution is or is not acid. In the mangold and sugar beet there is an apparent excess of dextrose over levulose which is due to the presence of glutamine, while in snowdrop, tropaeolum, and potato the presence of asparagine results in an apparent excess of levulose. In the first case the apparent excess of dextrose increases progressively from leaves through midribs and stalks as a consequence of the transfer of the impurity; in the leaves the dextrose-levulose ratio is in the neighborhood of unity; in the midribs and stalks it ranges from 2.5 to 10.0. The pentoses which are present in the alcoholic extract also affect the readings.

The author determined the proportions of the two sugars present in the three series discussed in the preceding paper, using the methods there employed. In the early morning there was found in young leaves a dextro-rotation still greater than that which would be observed if all the sugar present were dextrose. In older leaves there appeared to be a steady formation of a laevo-rotatory substance and a gradual transformation into a compound having still greater laevo-rotation. The author considers that this is manufacture of asparagine and transformation into aspartic acid. Both in the second and the third series there are three rises and three falls in the amount of apparent dextrose in 24 hours, this fact pointing to a regular and rhythmical variation in the rate of production of the optically active impurities.

The character of the optically active impurities in the upper portions of the stalks is quite different from that in the lower portions, as shown by the fact that when determinations of the sugars in the lower portions of the stalks are made simultaneously by polarization and reduction methods the polarization results are 40 per cent higher than those obtained by reduction, while on the upper portion of the same stalks the results by polarization are 85 per cent lower than the reduction figures. Hence the optically active substances interfering with the polarization are quite different in the two portions of the stalk, suggesting the optical behavior of d- and l-asparagine and d- and l-glutamine. Furthermore, there are two different optically active substances at different times during the 24 hours. For all these reasons we can at present obtain no true values for these sugars, and there is at least nothing to disprove the assumption that dextrose and levulose exist in the leaves and stalks as invert sugar, travel in approximately equal amounts to the roots, and there undergo recombination into saccharose.

In the third paper of the series DAVIS and SAWYER have applied similar methods of study to the potato as a typical plant forming starch in the leaves, paying especial attention to the mechanism of degradation of starch in the leaf, and have extended the study to a considerable number of plants, including turnip, sunflower, dahlia, carrot, grape, and others. They were able to find no maltose at any time, either during day or night, in plants storing much starch in the leaves, although more than 500 analyses by means of maltase-free yeasts were made. Hence the authors consider that BROWN and MORRIS were incorrect in their conclusion that diastatic formation of maltose and transfer as such occurs in the case of the leaf of *Tropaeolum*. Brown and Morris unquestionably had maltose present, as shown by the fact that they obtained the osazone and that there was an increase in the reduction of copper after treatment with maltase, but the authors consider that this result may be explained by the fact that the material used by Brown and Morris was subjected to preliminary drying in an oven. They regard the leaf as having a mixture of enzymes analogous to that found in *Aspergillus oryzae*, and that it is therefore able to split maltose rapidly and completely to dextrose. They destroyed all enzymes instantly by dropping the leaves as they were picked into a mixture of boiling alcohol and ammonia. As maltase is easily destroyed by heating to 55°, it was first to go out of action in Brown and Morris' oven-dried material, while other more heat-resistant enzymes went on forming maltose which was not split up, hence was found in the analysis. This hypothesis is borne out by the results; Davis and Sawyer invariably found more starch in the leaves than did Brown and Morris, the amount always exceeding the sum of starch plus maltose found by the last-named authors. Kluyver employed a biochemical method, using *Torula monosa* to ferment the hexoses only, *T. dattilla* to ferment the cane sugar and hexoses, leaving maltose, and found very small amounts of maltose.

The authors consequently believe that starch degradation goes immediately down to hexoses with no stop at maltose; that plants must reduce sugars to this form before they can be utilized; and that the fact that the sugar in leaf stalks is largely hexose is thus explained, as is the presence of invertase in almost all plant parts. Daish found maltase wherever starch is found in leaves, but believes it to be an intracellular enzyme occurring in close proximity to diastase, hence never found in the vessels of the stalks.

In the leaf saccharose is always greatly in excess of hexoses; in the stalks the reverse is always true. Hence saccharose must be the first product of photosynthesis and hexose a translocation form. The authors are led by unpublished work with a variety of other plants, such as sunflower, grape, and snowdrop, to the conclusion that this is the general situation with all plants regardless of the form in which final storage may occur. Like the potato, the plants just mentioned have two optically active impurities which are formed at different periods in the 24 hours, and hence have apparent large fluctuations in the dextrose-levulose ratio, which it is impossible to measure correctly by reason of their presence.

The authors found in the leaves considerable amounts of dextro-rotatory, water-soluble material which was not soluble starch or dextrin, which was greatest in amount between 4 and 8 P.M. Its period of greatest formation synchronizes with the high tide of saccharose and the period of most rapid starch formation, hence it seems to be intermediate between the hexoses and true starch. Starch is very rapidly reduced after sunset, then more slowly with the hexoses rising, while the starch rises again at dawn considerably before the hexoses show increase. The curves for hexose, starch, and this dextro-rotatory material are intimately related and indicate interconvertibility; the last-named substance may be a protein or a gum standing in causal relation to starch synthesis.

In the leaves the daily fluctuations of alcohol-soluble substances is through a range almost twice as great as that of total sugars. In the stalks the same is true, in which respect the potato is unlike the mangold. The dextrose-levulose ratio determinations are of little significance because of the presence of laevo-rotatory non-sugars, probably asparagine, but the authors regard them as being present in equal amounts as splitting products of saccharose. The polarization readings for saccharose were aberrant as in the mangold, by reason of the presence of impurities of the same character. Levulose apparently predominates in the leaves and dextrose in the stalks, by reason of the accumulation of dextro-rotatory stuffs in the latter, or possibly by reason of an actual excess due to the using up of levulose in tissue building. That this latter alternative is the correct one is indicated by the fact that the determinations of cane sugar by polarization and by reduction are in close agreement.—JOSEPH S. CALDWELL.

The Oenothera situation.—Three recent papers have cast some light on the perplexing *Oenothera* situation. One of the most serious objections to the mutation theory has been that mutants which have appeared under observation in artificial cultures have regularly been interfertile, while incipient species in nature are essentially intersterile. METZ and BRIDGES[4] have shown that mutants may be intersterile, describing two cases in *Drosophila*, each involving two mutants that either refuse to cross or else give sterile hybrids.

MULLER[5] has explained a curious case in *Drosophila*, which strikingly resembles the *Oenothera* situation. A certain race of *Drosophila* breeds practically true, and yet it is in a heterozygous condition. This paradox is explained by "balanced lethal factors," a given chromosome and its allelomorph each carrying lethal factors. When one of these factors is present in a zygote it brings death, but when both factors are present they are antagonistic in their action and the zygote develops into a mature individual. Thus the homozygotes, which are thrown off every generation, die in infancy, since they contain single lethal factors; only the heterozygotes survive, for in them alone are the lethal factors balanced and inactive. The result is that the heterozygous race seems to breed true. This balanced race, as we should expect, gives in crosses twin hybrids as in *Oenothera* crosses, while crossing two such balanced races in *Drosophila* gives multiple hybrids, as also occurs in *Oenothera*.

[4] METZ, C. W., and BRIDGES, C. B., Incompatibility of mutant races in *Drosophila*. Proc. Nat. Acad. Sci. 3:673–678. 1917.

[5] MULLER, HERMANN J., An *Oenothera*-like case in *Drosophila*. Proc. Nat. Acad. Sci. 3:619–626. 1917.